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Identification and expression profiling of chemosensory protein genes in *Galeruca daurica* (Coleoptera: Chrysomelidae)

LI Ling, ZHOU Yuan-Tao, TAN Yao, ZHOU Xiao-Rong, PANG Bao-Ping*

(Research Center for Grassland Entomology, Inner Mongolia Agricultural University, Hohhot 010019, China)

Abstract: 【Aim】*Galeruca daurica* is a new pest with outbreak status in grasslands of Inner Mongolia, northern China. The chemosensory proteins (CSPs) of insects are a class of small and water soluble proteins whose functions are to recognize and transport environmental chemical stimuli to receptors, participating in many aspects of insect behavior. The objective of this study is to identify the chemosensory protein genes and to analyze their expression profiles in *G. daurica*. 【Methods】The chemosensory protein genes in *G. daurica* were identified by screening the transcriptome data of *G. daurica* assembled in our laboratory, and qPCR was conducted to analyze their expression levels in different developmental stages (egg, 1st - 3rd instar larva, pupa and adult) and adult tissues (antenna, head with antennae removed, thorax, abdomen, leg and wing). 【Results】Ten chemosensory protein genes were identified from *G. daurica*, and named as *GdauCSP*1 - 10 (GenBank accession numbers: KY885471 - KY885480), respectively. The amino acid sequence identity between the 10 GdauCSPs ranges from 17.27% to 62.79% with a high divergence between each other. The results of Blast in NCBI showed that GdauCSPs have the highest amino acid sequence identity (90%) with PmacCSP from *Pyrrhalta maculicollis*. Phylogenetic analysis showed that four of the 10 GdauCSPs were firstly clustered into a clade with PmacCSPs from *P. maculicollis*. The qPCR results proved that the expression levels of *GdCSP*s were significantly different in different developmental stages, and five *GdCSP*s including *GdauCSP* 4 - 5, *GdauCSP*7 - 8, and *GdauCSP*10 had significantly higher expression levels in the adult stage than in other developmental stages while *GdCSP*2 had significantly higher expression level in the egg stage than in other developmental stages. *GdauCSP*s were also expressed in the head with antennae removed, thorax, abdomen, leg and wing besides in the antenna, and 10 *GdauCSP*s had different tissue expression profiles but five out of 10 *GdauCSP*s, including *GdauCSP*2, *GdauCSP*4, *GdauCSP*5, *GdauCSP*8 and *GdauCSP*9, had significantly higher expression levels in female antennae than in other tissues. 【Conclusion】These results suggest that GdauCSPs may play different roles in the development and chemosensory process of *G. daurica*. This study lays the necessary foundation for further research on the physiological function of CSPs and the molecular mechanism of chemical communication in *G. daurica*.

Key words: *Galeruca daurica*; chemosensory protein; expression profile; phylogenetic analysis; qPCR

1 INTRODUCTION

In diverse sensory systems of an insect, a highly sensitive chemoreception system plays an important role in its life. Insects contact and analyze the environmental information by chemoreceptors to ensure normal life activities, such as foraging, oviposition and mate recognition (Pilpel and Lancet, 1999; Pelosi *et al*., 2014). A variety of proteins are involved in chemical communication, including odorant binding proteins (OBPs), chemosensory proteins (CSPs), odorant receptors (ORs), ionotropic receptors (IRs), odorant-degrading proteins (ODEs), sensory neuron membrane proteins (SNMPs), and so on (Vogt and Riddiford, 1981; Buck, 1992; Pelosi *et al*., 2006; Benton *et al*., 2009). CSPs are a class of small soluble proteins expressed in the auxiliary cells of chemosensilla and secreted into the aqueous fluid surrounding the olfactory neurons (Pelosi *et al*., 2006, 2014). CSPs are characterized by the presence of four conserved cysteine residues forming two disulfide bridges (Angeli *et al*., 1999). CSPs are thought to be able to recognize and transport

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作者简介: 李玲, 女, 1990 年 10 月生, 内蒙古乌兰察布人, 博士研究生, 研究方向为昆虫生态与分子生物学, E-mail: 279165876@qq.com

* 通讯作者 Corresponding author, E-mail: pangbp@imau.edu.cn

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environmental chemical signals to receptors, a role similar to that of better-known OBPs in insect chemosensation (Sun *et al.*, 2014). CSPs were first discovered and named as olfactory-specific protein D (OS-D) in *Drosophila melanogaster* by McKenna *et al.* (1994). Some researches have shown that CSPs are not only broadly expressed in chemosensory organs, such as antennae, proboscises, maxillary palps, labial palps and tarsus (Angeli *et al.*, 1999; Jin *et al.*, 2006), but also expressed in non-chemosensory organs broadly, including legs, wings, pheromone glands and reproductive organs, implying that they may play a vital role in the process of growth and development in insects (Danty *et al.*, 1999; Pelosi *et al.*, 2006; Maleszka *et al.*, 2007; Guo *et al.*, 2011; Andersson *et al.*, 2013, 2014). For example, one CSP of *Plutella xylostella* is able to bind nonvolatile oviposition deterrents (Liu *et al.*, 2010). CSPs have been reported to be involved in larval development and brood pheromone transportation in *Apis mellifera* (Briand *et al.*, 2002; Forêt *et al.*, 2007).

Galeruca daurica (Coleoptera: Chrysomelidae) is an oligophagous insect, only feeding on the *Allium* plants, such as *A. mongolicum*, *A. ramosum* and *A. polyrrhizum* (Hao *et al.*, 2014, 2015). Extensive outbreaks of this pest since 2009 have caused great losses to pasture in grasslands of Inner Mongolia, northern China and the damage continues to increase, and it has become a major pest in the Inner Mongolian grasslands (Li *et al.*, 2014). Until now, the research on *G. daurica* has mainly focused on the physiological and ecological aspects, such as host plant selection (Hao *et al.*, 2014), life history (Hao *et al.*, 2015), cold hardiness (Li *et al.*, 2014, 2015; Gao *et al.*, 2015), genetic diversity (Zhang *et al.*, 2015), diapause (Zhou *et al.*, 2016b), and thermal requirement (Zhou *et al.*, 2016a). This pest only feeds on the species of *Allium* plants, implying an important role of olfaction in its search for specific host plants. Moreover, our previous investigation has shown that the adults begin to aggregate under cow dung, stone and grass for oversummering about one week after eclosion (Hao *et al.*, 2015). Therefore, olfaction may also play a key role in this aggregation behavior. However, little is known about the chemosensory mechanisms of this pest. In our previous study, we identified 29 putative OBP genes from the *G. daurica* transcriptome and analyzed their expression profiles (Li *et al.*, 2017). In this study, ten chemosensory protein genes of *G. daurica* were further identified from its transcriptome database assembled in our laboratory by using RNA-Seq (unpublished), and their expression profiles were examined. Our results lay a foundation for the functional characterization of the chemosensory proteins in this pest.

2 MATERIALS AND METHODS

2.1 Insects and sample collection

The 1st instar larvae of *G. daurica* were collected from Xilinhot, Inner Mongolia, China (43°54′53″N, 115°39′13″E) in 2015, and reared with *A. mongolicum* in incubators at 26 ± 1℃, a photoperiod of 16L∶8D and 60% – 80% relative humidity. For the expression profiling, 40 eggs, 20 1st instar larvae, 10 2nd instar larvae, three 3rd instar larvae and three pupae, and tissues including antennae, heads (without antennae), thoraxes, abdomens, legs and wings from six 3-day-old adults for each sex fed on *A. mongolicum* without mating were obtained for each replicate, transferred to Eppendorf tubes, frozen in liquid nitrogen and stored at – 80℃ until RNA extraction. Three replicates were conducted. All samples were collected on the 3rd day morning after oviposition or molting.

2.2 Identification and analysis of transcripts encoding putative chemosensory protein

We identified putative CSP genes by searching the transcriptome database of *G. daurica* adults assembled in our laboratory (unpublished). Putative CSP genes were searched by using "CSP" and "chemosensory protein" as the key words to screen the annotated sequences. Moreover, tBlastn was used to screen the transcriptome database and to identify the putative CSP genes using known CSP sequences of Chrysomelidae as "query". All putative CSP genes were manually confirmed using the BlastX program against the NR nucleotide database of the National Center for Biotechnology Information (NCBI) with a cut-off E-value = 10^{-5} (Li *et al.*, 2017). In order to confirm the sequences of putative CSPs, gene-specific primers (Table 1) were designed and used to clone the complete sequences of each candidate CSP gene, and the PCR products were sent to be sequenced. PCR was performed in a 50 μL reaction mixture, including cDNA 2 μL, forward primer (10 μmol/L) 2 μL, reverse primer (10 μmol/L) 2 μL, Premix Taq™ (V2.0 plus dye) (TaKaRa) 25 μL and RNase free water 19 μL. The reaction was conducted on a BIO-RAD T100™ Thermal Cycler (Bio-Rad, Hercules, CA, USA) using the following conditions: denaturation at 94℃ for 3 min followed by 30 cycles of 94℃ for 30 s, primer-specific temperature for 30 s, 72℃ for 1 min and a final extension at 72℃ for

10 min. The open reading frames (ORFs) of the putative CSP genes were predicted by using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/). The signal peptides of the amino acid sequences were predicted by using Signal IP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). The molecular weight and isoelectric point (pI) of the amino acid sequences were predicted by using DNAMAN V6 program. The similarity between the putative CSP genes was calculated by using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/).

2.3 Phylogenetic analysis of the CSPs in *G. daurica* and other insects

A phylogenetic tree was constructed to analyze the phylogenetic relationship of the CSPs between *G. daurica* and other insects, using the neighbor-joining method in MEGA6 (Tamura *et al.*, 2013). Branch support was assessed with 1 000 bootstrap replicates, and bootstrap support greater than 50% to be displayed. A total of 97 amino acid sequences, whose signal peptides were removed, of the CSPs from eight species of six orders, including Coleoptera: *Galeruca daurica* (10 GdauCSPs), *Pyrrhalta maculicollis* (10 PmacCSPs), *Tribolium castaneum* (19 TcasCSPs); Lepidoptera: *Bombyx mori* (16 BmorCSPs); Orthoptera: *Locusta migratoria* (5 LmigCSPs); Diptera: *Drosophila melanogaster* (20 DmelCSPs); Hymmenoptera: *Apis mellifera* (6 AmelCSPs); and Hemiptera: *Adelphocorid lineolatus* (11 AlinCSPs).

2.4 Expression profile analysis of *G. daurica* CSP genes by qPCR

Total RNA was extracted with TaKaRa Mini BEST Universal RNA Extraction Kit (TaKaRa, Dalian, China) from 40 eggs, 20 1st instar larvae, 10 2nd instar larvae, three 3rd instar larvae, three pupae, and six adults (3♀+3♂), and different tissues including antennae, heads without antennae, thoraxes, abdomens, legs and wings of six adults of each sex, which was as one biological replicate. The concentration and purity of total RNA were determined by micro spectrophotometer (NanoPhotometer® P330, Implen, Germany). The integrity of RNA was detected by agarose gel electrophoresis. cDNA was synthesized by using PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa) according to the manufacturer's instructions. Gene-specific primers of 10 CSPs were designed using Primer3 Input (http://primer3.ut.ee/). All primers used are listed in Table 1. The succinate dehydrogenase complex (SDHA) gene was used as a reference gene (Tan *et al.*, 2017). The qPCR was performed using the FTC-3000P Real-Time Quantitative Thermal Cycler (Funglyn Biotech, Canada) with BRYT Green® dye (GoTaq® qPCR Master Mix, Promega, America) as the fluorescence reporter for each elongation cycle. Experiments were

Table 1 Primer information

Primers	Sequence for CSP gene verification (5′-3′)	Sequence for qPCR (5′-3′)
SDHA-F		GGGAGACCACAATCTCCTCA
SDHA-R		AGCTGGTGCTCCTAAGTCCA
CSP1F	GAATTCATGAAGTTATCTCTTGTCG	AGCGGTAGTAGTAGTTTCTGCA
CSP1R	CTCGAGTTAGAGTTCAATTCCTTC	CCAACGCTTCCTTGAAAACC
CSP2F	GAATTCATGAATATATTACGTAACGC	ACCTGATACTTGCACCAAAGA
CSP2R	CTCGAGTTACAATGTATTTTTATATTCTT	GAGCATCGATTTTCTTCCACCA
CSP3F	GAATTCATGAATCGTTGGTGCATATC	TGCACTGTGCTCCAAAGAGT
CSP3R	CTCGAGTTATAGGGTGGGATC	ATCTTCTTCGTCGCAATCGT
CSP4F	GAATTCATGGGTTTAATACGATTAAT	TAAAAGGTGCTCACCGGAAG
CSP4R	CTCGAGTTAAGGACTATTTAAGAA	TCCTTGAGGATCAAACCTCTG
CSP5F	GAATTCATGTTTTCTTTGGTTGTGG	GGCTGCAGTTACCGAAAAAG
CSP5R	CTCGAGTTAGGTTTTGGTAATAGG	TCGCTGCATTTACTGCAATC
CSP6F	GAATTCATGAATTTTGTGTTTGTTCTTT	TGCACTAAAGATGGACAAGAGC
CSP6R	CTCGAGTTATAGTTCTAATCCTG	GCCTCTACTTCATTCCACCAA
CSP7F	GAATTCATGAAAACATTTCTGCTTATT	GCTGCAACCGGATCTTATGT
CSP7R	CTCGAGTTATGCTCTAATTTCTTT	TCGCTGCATTTTGAACAGTC
CSP8F	GAATTCATGAAATTAGCAATAGTTGTT	CAGCACCAGCTGATGAAAAA
CSP8R	CTCGAGTTACAGCTCTATTCC	TGCGTCCGGTAAAACTCTCT
CSP9F	GAATTCATGAATTTTTTCTGTGTGTC	AATGGTGGTGGCAGTTTCTG
CSP9R	CTCGAGCTATATTTTTTTGTCTTTATC	AGCACAACCTTGGTCCAAAC
CSP10F	GAATTCATGGTACCGTTAATCTG	GTTGGAACTGTCAGGGCAAT
CSP10R	CTCGAGTTAAACCTTCCTTTGA	TGTTGGTTCGGAAGGTTCAT

Table 2 General information of CSP genes identified from *Galeruca daurica* transcriptome

Genes	GenBank accession no.	ORF length (bp)	Molecular weight (kD)	Isoelectric point	Size of signal peptide (aa)	Best BlastX match					
						BLAST annotation	Score	Query cover (%)	*E*-value	Amino acid sequence identity (%)	GenBank accession no. of homogenous protein
*GdauCSP*1	KY885471	129	14.7536	5.36	18	Chemosensory protein 1 [*Pyrrhalta aenescens*]	183	89	1e-57	74	APC94294.1
*GdauCSP*2	KY885472	98	11.5254	7.91	–	Chemosensory protein 9 [*Pyrrhalta maculicollis*]	162	98	9e-50	72	APC94215.1
*GdauCSP*3	KY885473	136	15.8186	8.12	21	Chemosensory protein 9 [*Pyrrhalta aenescens*]	161	99	8e-49	72	APC94297.1
*GdauCSP*4	KY885474	124	14.5553	9.46	18	Chemosensory protein 6 [*Colaphellus bowringi*]	150	97	1e-44	52	ALR72520.1
*GdauCSP*5	KY885475	134	15.3239	7.09	16	Chemosensory protein 5 [*Pyrrhalta maculicollis*]	256	99	2e-86	90	APC94218.1
*GdauCSP*6	KY885476	130	15.1869	7.81	18	Chemosensory protein 3 [*Colaphellus bowringi*]	160	99	2e-48	58	ALR72517.1
*GdauCSP*7	KY885477	121	14.0759	9.09	18	Chemosensory protein 6, partial [*Pyrrhalta maculicollis*]	203	90	3e-65	89	APC94220.1
*GdauCSP*8	KY885478	130	14.8688	8.19	18	Chemosensory protein 2[*Pyrrhalta maculicollis*]	206	99	2e-66	80	APC94214.1
*GdauCSP*9	KY885479	119	13.5263	5.78	20	Chemosensory protein 8 [*Pyrrhalta aenescens*]	132	87	1e-37	56	APC94296.1
*GdauCSP*10	KY885480	273	30.0678	8.67	17	Chemosensory protein 3 [*Pyrrhalta maculicollis*]	303	98	2e-100	62	APC94216.1

–: Not detected.

performed in a 10 μL reaction mixture (1 μL cDNA, 0.2 μL 10 μmol/L forward primer, 0.2 μL 10 μmol/L reverse primer, 5 μL GoTaq ® qPCR Master Mix and 3.6 μL RNase Free Water). All reactions used the following conditions: denaturation at 95℃ for 10 min followed by 40 cycles of 95℃ for 15 s, 60℃ for 1 min and a dissociation at the end of each reaction. Three biological replicates and three technical replicates were conducted for each reaction to determine the reproducibility. The data analysis applied relative quantification by the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001). Differences in expression levels of each target gene among various tissues and developmental stages were compared using a one-way analysis of variance with a $P<0.05$ level of statistical significance, followed by Tukey's Honestly Significant Difference (HSD) test using SPSS 20.0.

3 RESULTS

3.1 Identification and analysis of putative CSP genes

From the transcriptome database, a total of 10 putative CSP genes were identified, and named as *GdauCSP*1 – 10 (GenBank accession no.: KY885471 – KY885480), and all had the full-length ORFs. The putative CSP genes identified in this study were then checked by PCR cloning and sequencing. The sequences obtained from positive clones share 94% – 100% nucleotide sequence identity with the corresponding sequences from the transcriptome. The general information of CSP genes identified from *G. daurica* transcriptome is listed in Table 2. The ORFs for these genes range in length from 98 to 273 bp with the molecular weights from 11.5254 to 30.0678 kD and isoelectric points from 5.36 to 9.46. Signal peptides consisting of 16 – 21 amino acid residues are present in all except GdauCSP2 which has no predicted signal peptide. The results of BlastX in NCBI showed that GdauCSPs show relatively high amino acid sequence identity with PmacCSPs from *Pyrrhalta maculicollis* and PaenCSPs from *P. aenescens*. The consensus of ten GdauCSPs varies from 17.27% (between GdauCSP5 and GdauCSP9) to 62.79% (between GdauCSP1 and GdauCSP8), showing high divergence (Table 3). By comparing the amino acid sequences, we found that GdauCSP1 – 10 have four conserved cysteines, which is consistent with the characteristics of the CSP family (Fig. 1).

Table 3 The consensus (%) of 10 GdauCSPs in amino acid sequence alignment

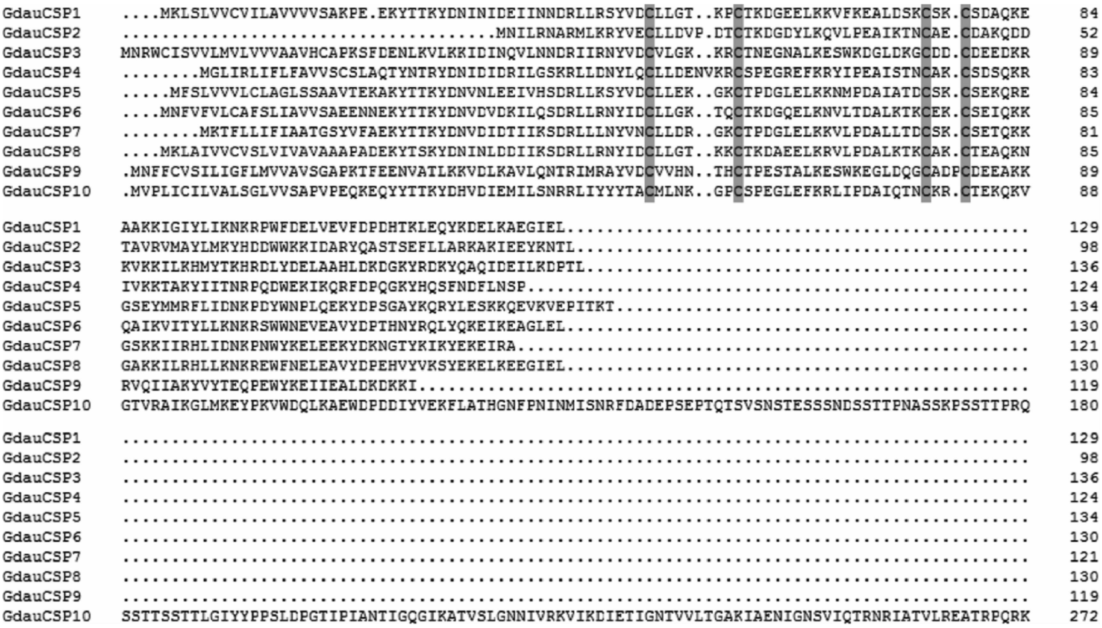
	GdauCSP1	GdauCSP2	GdauCSP3	GdauCSP4	GdauCSP5	GdauCSP6	GdauCSP7	GdauCSP8	GdauCSP9
GdauCSP2	29.67								
GdauCSP3	31.50	20.43							
GdauCSP4	35.54	30.34	27.73						
GdauCSP5	43.31	33.33	19.69	31.40					
GdauCSP6	51.94	37.36	30.40	32.79	42.19				
GdauCSP7	48.33	31.82	30.00	39.83	52.94	51.24			
GdauCSP8	62.79	32.97	32.00	34.43	43.75	60.00	54.55		
GdauCSP9	28.83	23.46	51.69	25.00	17.27	27.68	28.18	25.00	
GdauCSP10	28.35	28.87	28.03	37.50	34.35	30.47	34.45	28.91	20.18

3.2 Phylogenetic analysis of the CSPs in *Galeruca daurica* and other insects

A phylogenetic tree was generated to infer the relationships between the 10 CSPs of *G. daurica* and 87 CSPs of seven other insect species from six orders (Fig. 2). GdauCSPs were more scattered than those from other insects in the phylogenetic tree, although two CSPs formed a sister pair (GdauCSP3/GdauCSP9) with a bootstrap support of 90%. The genetic relationship was the closest between GdauCSPs and PmacCSPs, four of which were firstly clustered into the same branch, including GdauCSP7/PmacCSP6, GdauCSP10/PmacCSP3, GdauCSP2/PmacCSP9, and GdauCSP1/PmacCSP8.

3.3 Developmental stage-specific expression of *GdauCSP*s

The expression levels of *GdauCSP*s in different developmental stages were also different (Fig. 3), with the expression level in the 3rd instar larva as 1. Of the ten *GdauCSP*s, the expression levels of five *GdauCSP*s, including *GdauCSP*4 – 5, *GdauCSP*7 – 8, and *GdauCSP*10, were significantly higher in the adult stage than in other stages ($P<0.05$) (Fig. 3: D, E, G, H, J). *GdauCSP*1 had higher expression levels in the 1st and 2nd instar larva, which were approximately 2 800 times and 1 700 times as high as that in the 3rd instar larva, respectively, and its expression level in the 1st instar larva was significantly higher than that in the 2nd instar larva (Fig. 3: A). *GdauCSP*2 was exclusively expressed in the egg, with the relative expression level about 1 000 times as high as that in the 3rd instar larva (Fig. 3: B). The expression level of *GdauCSP*3 was the highest in the pupal stage, followed by the



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GdauCSP1    ....MKLSLVVCVILAVVVVSAKPE.EKYTTKYDNINIDEIINNDRLLRSYVDCLLGT..KPCTKDGEELKKVFKEALDSKCSK.CSDAQKE    84
GdauCSP2    ......................................MNILRNARMLKRYVECLLDVP.DTCTKDGDYLKQVLPEAIKTNCAE.CDAKQDD    52
GdauCSP3    MNRWCISVVLMVLVVVAAVHCAPKSFDENLKVLKKIDINQVLNNDRIIRNYVDCVLGK..KRCTNEGNALKESWKDGLDKGCDD.CDEEDKR    89
GdauCSP4    ........MGLIRLIFLFAVVSCSLAQTYNTRYDNIDIDRILGSKRLLDNYLQCLLDENVKRCSPEGREFKRYIPEAISTNCAK.CSDSQKR    83
GdauCSP5    .....MFSLVVVLCLAGLSSAAVTEKAKYTTKYDNVNLEEIVHSDRLLKSYVDCLLEK..GKCTPDGLELKKNMPDAIATDCSK.CSEKQRE    84
GdauCSP6    ....MNFVFVLCAFSLIAVVSAEENNEKYTTKYDNVDVDKILQSDRLLRNYIDCLLGK..TQCTKDGQELKNVLTDALKTKCEK.CSEIQKK    85
GdauCSP7    ........MKTFLLIFIAATGSYVFAEKYTTKYDNVDIDTIIKSDRLLLNYVNCLLDR..GKCTPDGLELKKVLPDALLTDCSK.CSETQKK    81
GdauCSP8    ....MKLAIVVCVSLVIVAVAAAPADEKYTSKYDNINLDDIIKSDRLLRNYIDCLLGT..KKCTKDAEELKRVLPDALKTKCAK.CTEAQKN    85
GdauCSP9    .MNFFCVSILIGFLMVVAVSGAPKTFEENVATLKKVDLKAVLQNTRIMRAYVDCVVHN..THCTPESTALKESWKEGLDQGCADPCDEEAKK    89
GdauCSP10   .MVPLICILVALSGLVVSAPVPEQKEQYYTTKYDHVDIEMILSNRRLIYYYTACMLNK..GPCSPEGLEFKRLIPDAIQTNCKR.CTEKQKV    88

GdauCSP1    AAKKIGIYLIKNKRPWFDELVEVFDPDHTKLEQYKDELKAEGIEL...............................................   129
GdauCSP2    TAVRVMAYLMKYHDDWWKKIDARYQASTSEFLLARKAKIEEYKNTL..............................................    98
GdauCSP3    KVKKILKHMYTKHRDLYDELAAHLDKDGKYRDKYQAQIDEILKDPTL.............................................   136
GdauCSP4    IVKKTAKYIITNRPQDWEKIKQRFDPQGKYHQSFNDFLNSP...................................................   124
GdauCSP5    GSEYMMRFLIDNKPDYWNPLQEKYDPSGAYKQRYLESKKQEVKVEPITKT..........................................   134
GdauCSP6    QAIKVITYLLKNKRSWWNEVEAVYDPTHNYRQLYQKEIKEAGLEL...............................................   130
GdauCSP7    GSKKIIRHLIDNKPNWYKELEEKYDKNGTYKIKYEKEIRA....................................................   121
GdauCSP8    GAKKILRHLLKNKREWFNELEAVYDPEHVYVKSYEKELKEEGIEL...............................................   130
GdauCSP9    RVQIIAKYVYTEQPEWYKEIIEALDKDKKI..............................................................   119
GdauCSP10   GTVRAIKGLMKEYPKVWDQLKAEWDPDDIYVEKFLATHGNFPNINMISNRFDADEPSEPTQTSVSNSTESSSNDSSTTPNASSKPSSTTPRQ   180

GdauCSP1    ............................................................................................   129
GdauCSP2    ............................................................................................    98
GdauCSP3    ............................................................................................   136
GdauCSP4    ............................................................................................   124
GdauCSP5    ............................................................................................   134
GdauCSP6    ............................................................................................   130
GdauCSP7    ............................................................................................   121
GdauCSP8    ............................................................................................   130
GdauCSP9    ............................................................................................   119
GdauCSP10   SSTTSSTTLGIYYPPSLDPGTIPIANTIGQGIKATVSLGNNIVRKVIKDIETIGNTVVLTGAKIAENIGNSVIQTRNRIATVLREATRPQRK   272
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Fig. 1　Amino acid sequence alignment of 10 putative CSPs in *Galeruca daurica*

Gray boxes show conserved cysteines.

3rd instar larva (Fig. 3: C). *GdauCSP*6 was not expressed in the egg and pupal stages (Fig. 3: F).

3.4　Tissue-specific expression analysis of *GdauCSP*s

In order to explore the potential function, tissue-specific expression of *GdauCSP*s was performed by qPCR (Fig. 4), with the expression level in male antennae as 1. The results showed that *GdauCSP*1 – 10 were expressed at different levels in male and female adult antennae of *G. daurica*. The expression levels of seven *GdauCSP*s including *GdauCSP*2 – 5 and *GdauCSP*8 – 10, were significantly higher in female antennae than in male antennae ($P < 0.05$) (Fig. 4: B – E, H – J). Three *GdauCSP*s (*GdauCSP*1, *GdauCSP*6, and *GdauCSP*7) had no significant difference in expression levels between males and females ($P > 0.05$) (Fig. 4: A, F, G). Besides in the antenna, *GdauCSP*s were also expressed in other tissues, but five *GdauCSP*s, *i.e.*, *GdauCSP*2, 4, 5, 8 and 9, had significantly higher expression levels in female antennae higher than in other tissues (Fig. 4: B, D, E, H, I). The expression of *GdauCSP*1 was significantly higher in the head than in other tissues, and was 140 times as high as that in male antennae (Fig. 4: A). *GdauCSP*5 was primarily expressed in the antenna, and had lower expression levels in other tissues (Fig. 4: E). *GdauCSP*3, *GdauCSP*7, and *GdauCSP*10 had relatively high expression levels in the wing (Fig. 4: C, G, J). Especially, *GdauCSP*6 was found exclusively enriched in the abdomens of female adults, with the expression level of approximately 1 600 times as high as that in male antennae (Fig. 4: F).

4　DISCUSSION

In recent years, numerous chemosensory genes have been identified from coleopteran insects with the development of the genome and transcriptome sequencing technology, such as *Ips typographus* and *Dendroctonus ponderosae* (Andersson *et al*., 2013), *Monochamus alternatus* and *Dastarcus helophoroides* (Wang *et al*., 2014), *Colaphellus bowringi* (Li *et al*., 2015), *Rhynchophorus ferrugineus* (Antony *et al*., 2016), *Ambrostoma quadriimpressum* (Wang *et al*., 2016), *Pyrrhalta maculicollis* and *P. aenescens* (Zhang *et al*., 2016). In this study, we identified 10 CSP genes from the *G. daurica* transcriptome, all of which are reported for the first time. The number of CSP genes identified in *G. daurica* is almost the same as that in other insects in Coleoptera except *T. castaneum* (Tribolium Genome Sequencing Consortium, 2008). This supported the reliability of the transcriptome data of *G. daurica*. While the number of *GdauCSP*s was clearly lower than 20 which was the number of CSP genes identified in the genome of *T. castaneum*, implying that some additional *G. daurica* CSP genes with lower expression levels are waiting to be identified by other techniques.

The results of BlastX in NCBI showed that GdauCSPs have relatively high amino acid sequence identity with PmacCSPs from *P. maculicollis* and PaenCSPs from *P. aenescens*, and this is consistent with their taxonomic position. *G. daurica*, *P. maculicollis* and *P. aenescens* are the members of Chrysomelidae (Coleoptera), thus it is reasonable that they have quite similar chemosensory systems.

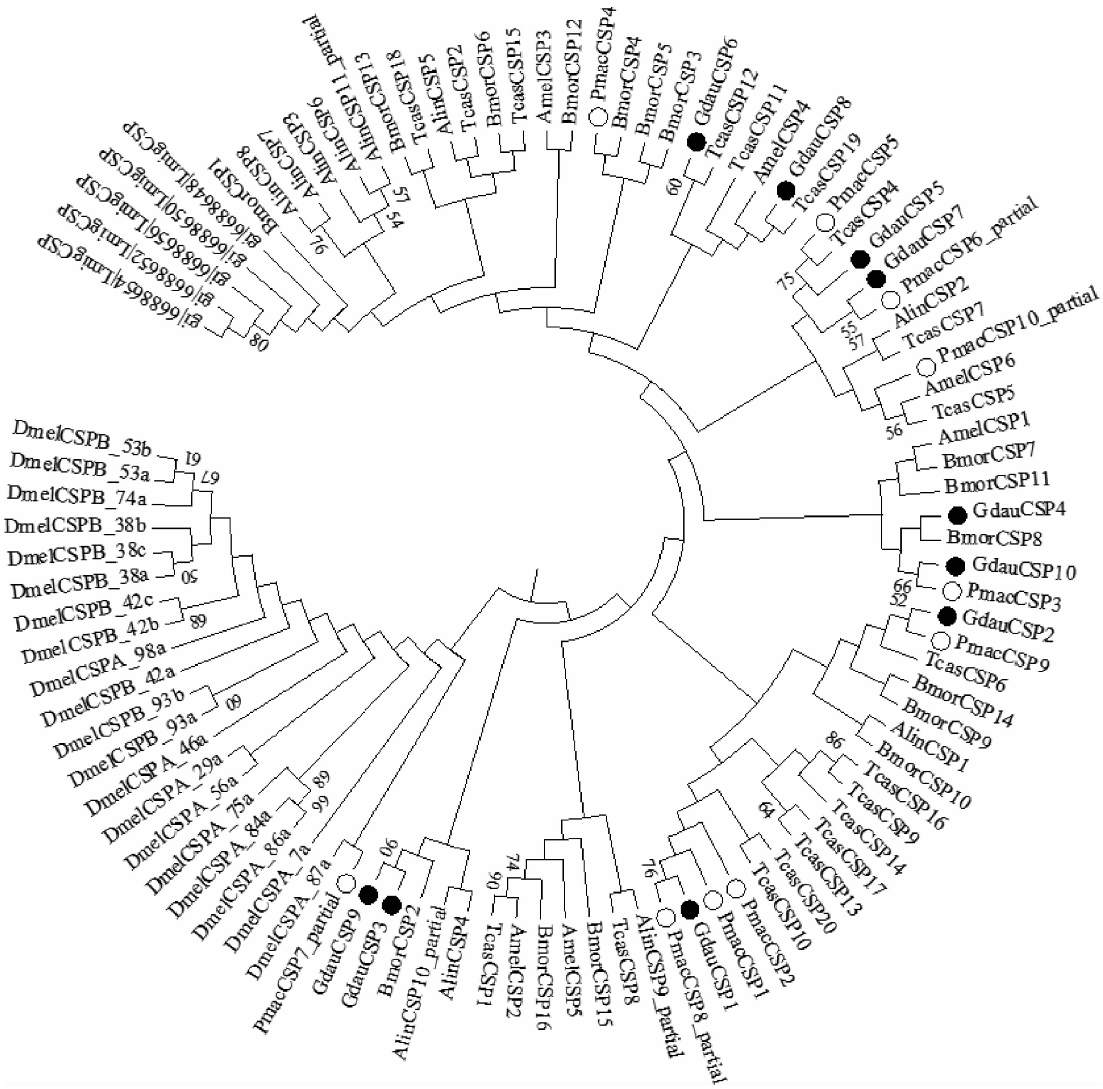

Fig. 2 A phylogenetic tree based on the amino acid sequences of CSPs from *Galeruca daurica* and other insects

Phylogenetic tree was constructed by neighbor-joining method using the program MEGA 6.0 with 1 000 bootstrap replications. Bootstrap values >50% are shown. Black dots indicate *Galeruca daurica* CSPs, while circles indicate *Pyrrhalta maculicollis* CSPs. Amel: *Apis mellifera* (GenBank accession no: ABH88169.1 – ABH88174.1); Dmel: *Drosophila melanogaster* (GenBank accession no.: NP_572395.2, NP_610061.2, NP_649035.2, NP_650280.1, NP_650970.2, NP_652269.3, NP_732641.2, NP_788004.1, NP_995735.1, NP_995736.1, NP_995763.1 – NP_995765.1, NP_001014530.1, NP_001014531.1, NP_001027134.1, NP_001027150.1, NP_001027174.1, NP_001027399.1, NP_001027439.1); Bmor: *Bombyx mori* (GenBank accession no.: AAM34276.1, AAM34275.1, BAF34351.1 – BAF34359.1, ABH88205.1 – ABH88209.1); Tcas: *Tribolium castaneum* (GenBank accession no.: ABH88175.1 – ABH88193.1); Alin: *Adelphocoris lineolatus* (GenBank accession no.: ACZ58019.1 – ACZ58026.1, AMD02858.1 – AMD02860.1); Lmig: *Locusta migratoria* (GenBank accession no.: CAB65177.1 – CAB65181.1); Pmac: *Pyrrhalta maculicollis* (GenBank accession no.: APC94212.1 – APC94221.1); Gdau: *Galeruca daurica* (GenBank accession no.: ARM20137.1 – ARM20146.1).

The consensus of ten GdauCSPs varies in a larger range, and the distributions are more scattered in the phylogenetic tree, indicating that there is high divergence between GdauCSPs. The neighbor-joining tree showed that most branches of the bootstrap support were less than 50%, indicating that there is a highly dynamic evolutionary pattern of CSPs among different insect species, which is consistent with the evolutionary process of different orders of arthropods (Kulmuni and Havukainen, 2013). Especially, the phylogeny showed that four pairs of GdauCSPs and PmacCSPs (GdauCSP1/PmacCSP8, GdauCSP7/PmacCSP6, GdauCSP10/PmacCSP3, and GdauCSP2/PmacCSP9) were clustered into the same clades with bootstrap support ranging from 56% to 97%, indicating that they could be homologous and may have similar functions.

Identification and expression profiling of chemosensory genes are of primary importance for exploring their functions and the mechanisms of insect olfaction. The expression profiles showed that *GdauCSP*s were not only expressed in the adult antennae, but broadly expressed in the leg, wing, head, thorax, and abdomen, which is in agreement with the previous reports that *CSP*s were also expressed in non-chemosensory organs besides

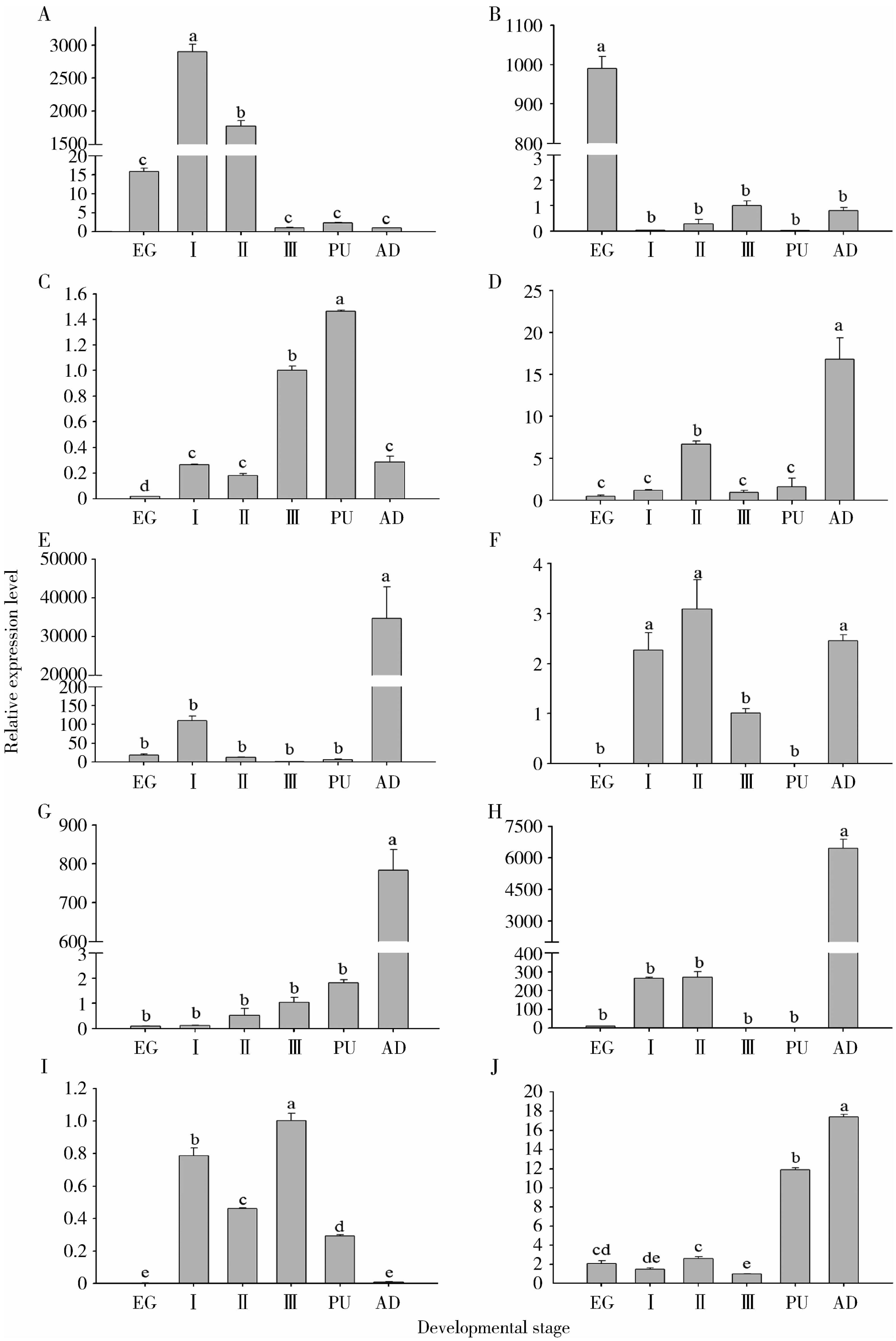

Fig. 3　Expression profiles of 10 CSP genes *GdauCSP*1 – 10 (A – J) in different developmental stages of *Galeruca daurica*
EG: Egg; Ⅰ: 1st instar larva; Ⅱ: 2nd instar larva; Ⅲ: 3rd instar larva; PU: Pupa; AD: Adult. The bars indicate the standard error of the mean for three independent experiments, and different letters above each bar denote significant differences (Tukey's test, $P < 0.05$).

chemosensory organs (Nagnan-Le Meillour *et al.*, 2000; Picimbon *et al.*, 2000; Kim *et al.*, 2003; Gong *et al.*, 2007; Liu *et al.*, 2010; Zhang *et al.*, 2013), suggesting that they may participate in other physiological processes beyond chemoreception (Kitabayashi *et al.*, 1998). The expression levels of *GdauCSP*3 and *GdauCSP*7 were significantly higher in the wing than in other tissues, suggesting that they may have other functions besides olfaction. *GdauCSP*5 had significantly higher expression level in the antenna than in other tissues, and it may be associated with sensing chemical stimulus.

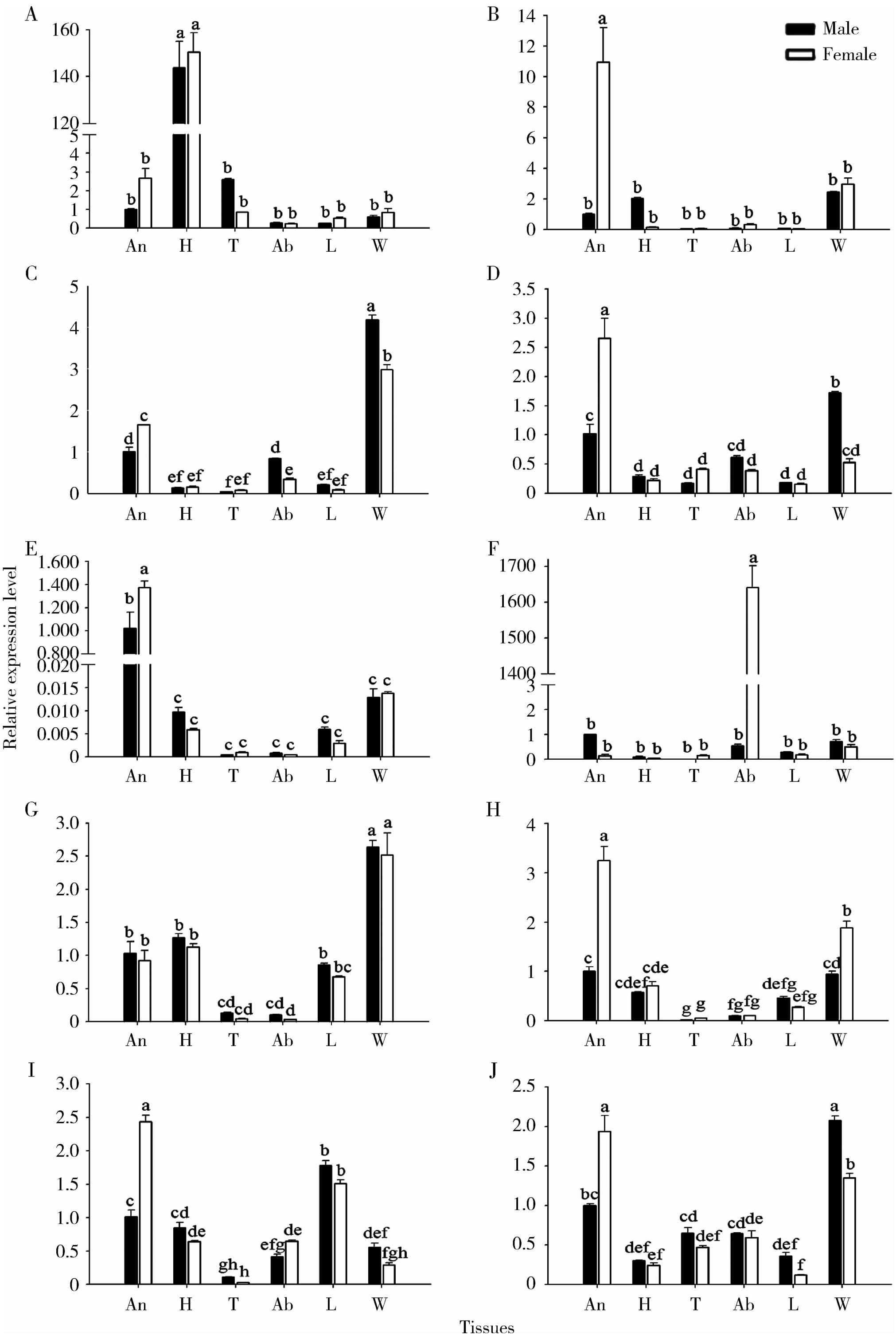

Fig. 4 Tissue-specific expression profiles of 10 CSP genes *GdauCSP*1 – 10 (A – J) in *Galeruca daurica* adults

An: Antennae; H: Head (with antennae removed); T: Thorax; Ab: Abdomen; L: Leg; W: Wing. The bars indicate the standard error of the mean for three independent experiments, and different letters above each bar denote significant differences (Tukey's test, $P < 0.05$).

Noticeably, *GdauCSP*6 was found exclusively enriched in the abdomens of female adults, implying that it may play roles in female adults such as hormone transportation, ovary development, oviposition, and so on.

The developmental expression profiles showed that *GdauCSP*1 had relatively higher expression levels in the 1st and 2nd instar larva, and moreover, the expression level was significantly higher in the 1st instar larva than in the 2nd instar larva, suggesting that it may play an important role in the transportation of larval pheromone. Briand *et al.* (2002) obtained similar results. *GdauCSP*2 was found to be specifically expressed in the egg, and it may be involved in the normal development of the embryo. Maleszka *et al.* (2007) found that silencing *AmelCSP*5 in the egg of *Apis mellifera* caused developmental malformation of the head. The CSP genes in *G. daurica* had diverse expression profiles regarding tissues or developmental stages, suggesting that they may play different roles in the process of growth and development, which need to be further explored.

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沙葱萤叶甲化学感受蛋白基因的鉴定及表达谱分析

李玲, 周渊涛, 谭瑶, 周晓榕, 庞保平*

(内蒙古农业大学草原昆虫研究中心, 呼和浩特 010019)

摘要:【目的】沙葱萤叶甲 *Galeruca daurica* 是一种在内蒙古草原上爆发成灾的新害虫。昆虫的化学感受蛋白(CSPs)是一类水溶性小分子蛋白,其主要功能是识别和传导环境中的化学刺激至受体,参与众多昆虫行为。本研究旨在鉴定沙葱萤叶甲化学感受蛋白基因,并对其表达谱进行分析。【方法】通过筛选本实验室组装的沙葱萤叶甲转录组鉴定沙葱萤叶甲化学感受蛋白基因;采用实时荧光定量 PCR 方法分析其在沙葱萤叶甲不同发育阶段(卵、1 – 3 龄幼虫、蛹和成虫)和成虫组织[触角、头(去触角)、胸、腹、足和翅]中的表达水平。【结果】鉴定出 10 个化学感受蛋白基因,将其命名为 *GdauCSP*1 – 10 (GenBank 登录号: KY885471 – KY885480)。10 条编码蛋白的氨基酸序列一致性范围为 17.27% ~ 62.79%,彼此间分化程度较高。通过 NCBI 的 Blast 比对结果显示, GdauCSPs 与榆黄毛萤叶甲 *Pyrrhalta maculicollis* 的 PmacCSP 氨基酸序列一致性最高,为 90%。系统发育分析表明,4 种 GdauCSPs 首先与榆黄毛萤叶甲的 PmacCSPs 聚为一支。实时荧光定量 PCR 结果显示, *GdCSPs* 在不同发育阶段中的表达量有显著差异, *GdauCSP*4 – 5, *GdauCSP*7 – 8 和 *GdauCSP*10 5 个 *GdCSPs* 在成虫中的表达量显著高于其他发育阶段,而 *GdCSP*2 在卵中的表达量显著高于其他发育阶段; *GdCSPs* 不仅表达于成虫触角中,在头(去触角)、胸、腹、足、翅等其他部位也有表达,且 10 个 *GdCSPs* 在沙葱萤叶甲成虫各组织中有着不同的表达谱,其中 *GdauCSP*2, *GdauCSP*4, *GdauCSP*5, *GdauCSP*8 和 *GdauCSP*9 5 个 *GdCSPs* 在雌成虫触角中的表达量显著高于其他组织。【结论】结果提示化学感受蛋白在沙葱萤叶甲的生长发育和化学感受过程中可能起着不同的作用。本研究为进一步研究沙葱萤叶甲化学感受蛋白的生理功能及化学通讯的分子机理奠定了必要的基础。

关键词: 沙葱萤叶甲; 化学感受蛋白; 表达谱; 系统发育分析; qPCR

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